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PROSTAGLANDIN E_2 IS INVOLVED IN ADRENOCORTICOTROPHIC HORMONE RELEASE DURING SWIMMING EXERCISE IN RATS

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SUMMARY

1. We found that adrenocorticotrophic hormone (ACTH) release in rats induced by acute swimming exercise or by an intravenous injection of human recombinant interleukin-1β (IL-1β) was significantly attenuated after chronic exercise.

2. Since involvement of prostaglandins in the ACTH response induced by IL-1 is well known, we investigated the effect of indomethacin, an inhibitor of prostaglandin synthesis, on the ACTH response induced in rats by acute swimming exercise. Pretreatment with an intravenous injection of indomethacin significantly suppressed the ACTH response induced by exercise. The effect of indomethacin (1 and 10 mg/kg) on the ACTH response was dose-dependent.

3. The effect of chronic exercise on the exercise-induced changes in the plasma concentration of prostaglandin E_2 was investigated. The plasma concentration of prostaglandin E_2 significantly increased after acute exercise in both the control and the chronically exercised rats. However, the increase in the plasma level of prostaglandin E_2 was significantly smaller in the chronically exercised group than in the control group.

4. Intravenous injections of prostaglandin E_2 produced dose-dependent increases in the plasma concentration of ACTH in rats.

5. The present results suggest that an increase in prostaglandin E_2 levels in plasma is involved in the development of the ACTH response induced by exercise.

INTRODUCTION

It is well known that during stressful conditions such as exercise or infection, plasma levels of ACTH increase significantly. The mechanism of the ACTH response is thought to be through hypothalamic neuroendocrine cells that secrete corticotrophin-releasing factor (CRF) into the hypophyseal portal vein. CRF subsequently stimulates the pituitary cells to secrete ACTH (Vale, Spiess, Rivier & Rivier, 1981; Rivier & Plotsky, 1986). However, the triggers that induce the ACTH response during stress still remain unknown.

Under infectious or inflammatory conditions, several kinds of cytokines are released from circulating and reticuloendothelial leucocytes. In recent years, it has

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been shown that some cytokines such as interleukin-1 (IL-1) (Besedovsky, del Rey, Sorkin & Dinarello, 1986), interleukin-6 (Naitoh, Fukuta, Tominaga, Nakai, Tamai, Mori & Imura, 1988) and tumour necrosis factor (Sharp, Matta, Peterson, Newton, Chao & McAllen, 1989) play an important role in inducing the ACTH response under infectious or inflammatory conditions. The ACTH response induced by IL-1 appears to occur by the activation of the arachidonic acid cascade system to cause the synthesis and release of prostaglandins. Prostaglandins may then act on the hypothalamus to secrete CRF, which, in turn, stimulates the secretion of ACTH at the adenohypophysis (Watanabe, Morimoto, Sakata & Murakami, 1990).

In the present study, we found that the ACTH response induced by intravenous IL-1 was significantly attenuated after chronic exercise. Since the ACTH response induced by acute exercise was also reduced after chronic exercise, we hypothesized that there is a cross-adaptation in the ACTH response to these two different kinds of stress, exercise and infection. Therefore, we speculated that during the stressful conditions of exercise or infection, similar mechanisms are involved in the ACTH response. In an earlier report, we showed that pre-treatment with indomethacin, an inhibitor of prostaglandin synthesis, significantly suppressed the ACTH response induced by IL-1 (Morimoto, Murakami, Nakamori, Sakata & Watanabe, 1989; Watanabe *et al.* 1990). Therefore, in the present study, we decided to study the possible role of prostaglandins in the exercise-induced ACTH response.

METHODS

Male albino rats (Wistar strain) weighing 240–270 g were used in this study. Rats were acclimated to a 12 h light–dark cycle (light on at 07.00 h and off at 19.00 h) under a room temperature of 26 ± 1 °C. The exercise performed in this study was swimming. The present study had been permitted by the Animal Care Committee of our medical school, and the chairman and members of the Committee had inspected the experimental procedure. During exercise, animals swam in a small round pool (50 cm diameter). The water of the pool was circulated and its temperature was maintained at 36 °C. Previous reports have demonstrated that rats do not show marked changes in rectal temperature (Ostman, Sjostrand & Swedin, 1972) and that they can swim for 60 h in water at 36 °C (Dawson & Horvath, 1970), indicating that the water temperature of 36 °C is suitable for rats to swim in. During the exercise, the rats' conditions were carefully watched, and when the rats showed symptoms of fatigue, the exercise was stopped.

In the chronically exercised group, the swimming exercise was performed for 1 h per day, and 5 days a week. This was done for 4 weeks. During exercise, animals wore a weight of 5 g attached to their necks and swam in the small round pool. Based on previous studies (Dawson & Horvath, 1970), a weight of 5 g (about 2% of the body weight) is not considered to be heavy. In the control group, to control for the psychological effect of immersion in the water, animals swam without the weight for 3 min per day and 5 days a week. This was also carried out for 4 weeks. Immediately after swimming, rats were rubbed with a towel and wet fur was dried by a hair dryer. The body weight of the animals in both the control and the chronically exercised groups was measured every day. During chronic exercise, there were no significant differences in the mean values of the total body weight between the control and the chronically exercised groups.

Human recombinant interleukin-1β (IL-1β) was supplied by the Otsuka Pharmaceutical Co. Ltd. For injection, the recombinant IL-1β was dissolved in sterile saline at a concentration of 2 μg/ml. These solutions were divided into several vials and stored at -40 °C until use. We used each vial within 2 days after thawing to avoid repeated freezing and thawing. Indomethacin was dissolved in saline containing 4% sodium bicarbonate and 0·5% ethanol at a concentration of 10 or 1 mg/ml. We used saline containing 4% sodium bicarbonate and 0·5% ethanol as a control vehicle for indomethacin. Prostaglandin E_2 was dissolved in saline at a concentration of 1 or

0·1 mg/ml. The volume of intravenous injection was always 1 ml/kg, including the volume of the control vehicle.

For intravenous injection or blood sampling, all rats had been previously catheterized with a cannula. Polyvinyl tubing was inserted into a superior caval vein of rats under general anaesthesia (sodium pentobarbitone, 40 mg/kg, I.P.) using the method of transjugular cannulation previously described (Harms & Ojeda, 1974). To measure the plasma concentration of prostaglandin E_2, about 1·0 ml of blood was withdrawn through the cannula. To measure the plasma concentration of ACTH, about 0·5 ml of blood was withdrawn. All experiments were carried out between 10.00 and 12.00 h.

Blood was collected into polyethylene tubes containing EDTA solution (1 mg/ml blood) to measure the ACTH concentration or into test-tubes containing indomethacin (0·5 mg/ml blood) to measure the prostaglandin E_2 concentration. Blood was centrifuged at 2000 r.p.m. for 15 min at 4 °C and the plasma was collected into test-tubes and stored at −20 °C. To determine the ACTH and prostaglandin E_2 concentrations, a radioimmunoassay for ACTH or prostaglandin E_2 was performed, using commercial radioimmunoassay kits (Diagnostic Product Corporation, USA; Amersham, USA).

The data were analysed for statistical significance by Student's *t* test or by ANOVA.

RESULTS

Figure 1*A* shows changes in the plasma concentration of ACTH after swimming. In both the control and the chronically exercised groups, the plasma concentration of ACTH significantly increased 20 min after the end of swimming for 30 min, as compared with the ACTH concentration 30 min before swimming. However, it is noted that the increased plasma concentration after swimming in the chronically exercised group was significantly smaller than that in the control group, although no significant difference in the plasma ACTH concentration before the start of swimming was observed in the two groups. Figure 1*B* shows changes in the plasma level of ACTH after intravenous injection of IL-β (2 μg/kg). In the control group, the plasma concentration of ACTH significantly increased 30 and 90 min after the injection of IL-1β. In contrast, in the chronically exercised group, the ACTH concentration significantly increased 30 min after injection of IL-1β, but 90 min after the injection it had returned to a level near that which was measured before injection. Furthermore, the increased plasma concentration of ACTH following IL-1β injection in the chronically exercised group was significantly smaller than that seen in the control group.

Figure 2 shows the effect of indomethacin on the ACTH response induced by swimming. The plasma concentration of ACTH significantly increased 20 min after the end of 30 min of swimming. However, pre-treatment with an intravenous injection of indomethacin 15 min before the start of swimming significantly suppressed the increase in the plasma levels of ACTH induced by exercise. The effects of indomethacin on the ACTH response were dose-dependent.

Figure 3 shows changes in the plasma concentrations of prostaglandin E_2 after swimming in the control and the chronically exercised groups. The plasma levels of prostaglandin E_2 increased significantly in both groups after swimming. However, the increases in the levels of prostaglandin E_2 were significantly smaller in the chronically exercised group than in the control group.

Figure 4 shows the effect of intravenous injection of prostaglandin E_2 on the plasma concentration of ACTH. As shown in Fig. 4, 20 min after intravenous

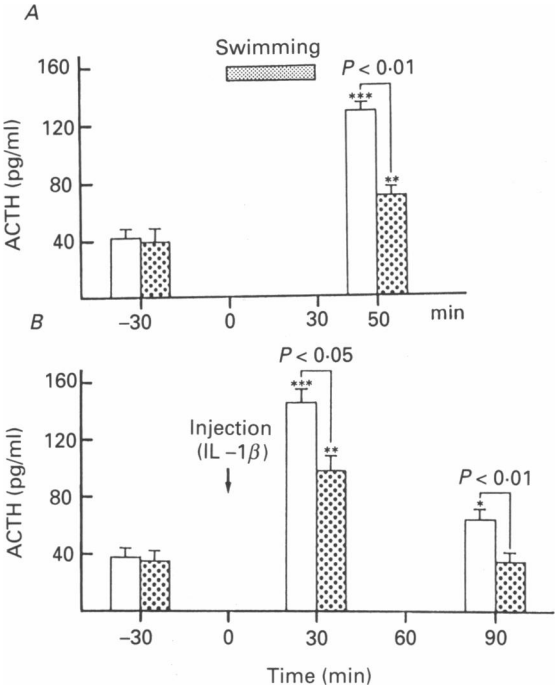

Fig. 1. *A*, mean changes (±S.E.M.) in the plasma concentration of ACTH in the control (□, $n = 7$) and the chronically exercised (▨, $n = 7$) groups 20 min after the end of swimming for 30 min. $^{**}P < 0{\cdot}01$; $^{***}P < 0{\cdot}001$. *B*, mean changes (±S.E.M.) in the plasma concentration of ACTH in the control (□, $n = 7$) and the chronically exercised (▨, $n = 7$) groups 30 and 90 min after intravenous injection of IL-1β (2 μg/kg). $^{*}P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$; $^{***}P < 0{\cdot}001$.

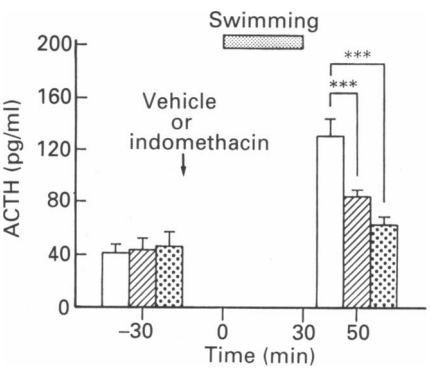

Fig. 2. Mean changes (±S.E.M.) in the plasma concentration of ACTH in the same group of eight rats, 20 min after the end of swimming for 30 min with systemic pre-treatment of indomethacin 15 min before the start of swimming. $^{***}P < 0{\cdot}001$. □, vehicle; ▨, 1 mg/kg indomethacin; ▩, 10 mg/kg indomethacin.

injection of prostaglandin E_2, the plasma ACTH concentration increased significantly. The effects of intravenous injections of prostaglandin E_2 on the ACTH response were dose-dependent.

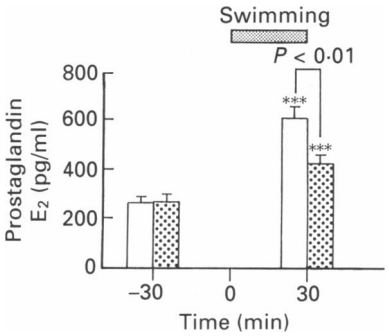

Fig. 3. Mean changes (±S.E.M.) in the plasma concentration of prostaglandin E_2 in the control (□, $n = 9$) and the chronically exercised (▨, $n = 9$) groups immediately after the end of swimming for 30 min. ***$P < 0{\cdot}001$.

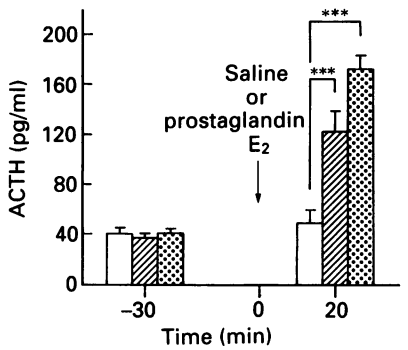

Fig. 4. Mean changes (±S.E.M.) in the plasma concentration of ACTH in the same group of six rats 20 min after intravenous injections of prostaglandin E_2. ***$P < 0{\cdot}001$. □, saline; ▨, 0·1 mg/kg prostaglandin E_2; ▩, 1 mg/kg prostaglandin E_2.

DISCUSSION

The present results show that circulating prostaglandins play an important role in the development of the ACTH response induced by acute exercise. We have shown that an inhibitor of prostaglandin synthesis significantly suppressed the increase in the plasma concentration of ACTH after exercise. Furthermore, we confirmed that, after exercise, the plasma level of prostaglandin E_2 increased significantly, along with the plasma ACTH concentration. These results indicate that the plasma prostaglandin E_2 concentration increases during exercise and support the hypothesis that prostaglandin E_2 causes the ACTH response. This is further established by the present results showing that exogenous prostaglandin E_2 injected intravenously increased the plasma level of ACTH in a dose-dependent manner. However, the dosages of prostaglandin E_2 (1 or 0·1 mg/kg) used in the present study seem to be relatively high. It is well known that about 90% of all the prostaglandin E_2 in blood is metabolized during each pass through the pulmonary circulation (Piper, Vane & Wyllie, 1970). Although it is reported that prostaglandin E_2 injected intravenously is able to enter the brain across the blood–brain barrier (Dascombe & Milton, 1979;

Eguchi, Hayashi, Urade, Ito & Hayaishi, 1988), prostaglandin E_2 injected as a bolus is considered to be metabolized very quickly. Furthermore, during swimming the plasma concentration of prostaglandin E_2 may increase throughout the exercise period. Since we gave a bolus injection of prostaglandin E_2 instead of continuous infusion, the doses of prostaglandin E_2 used in this study were not especially high. Therefore, the finding in the present study that intravenous injection of prostaglandin E_2 induced significant increases in plasma concentration of ACTH strongly supports the idea that plasma prostaglandin E_2 may be responsible for the ACTH response induced by acute exercise.

Recently, we have shown that an intrahypothalamic injection of a small dose of prostaglandin E_2 (25 ng) induces the secretion of ACTH (Morimoto *et al.* 1989; Watanabe *et al.* 1990) and that the ACTH response induced by intrahypothalamic prostaglandin E_2 is significantly suppressed by pre-treatment with an intravenous anti-CRF antibody (Watanabe *et al.* 1990). Therefore it is likely that the increased levels of prostaglandin E_2 in the circulation during exercise act on the hypothalamus. This is supported by the fact that circulating prostaglandin E_2 is able to enter the brain by crossing the blood–brain barrier (Dascombe & Milton, 1979; Eguchi *et al.* 1988). We believe that hypothalamic prostaglandins subsequently stimulate CRF secretion and that CRF released in the hypophyseal portal vein then stimulates the secretion of ACTH at the adenohypophysis.

In addition, the present study showed that the increase in the plasma level of prostaglandin induced by acute exercise is significantly suppressed after chronic exercise. The ACTH response to acute exercise was also reduced after chronic exercise. This seems to suggest that, after chronic exercise, animals adapt to physical stress by reducing prostaglandin synthesis and/or by enhancing the degradation of prostaglandin.

The present study shows that the ACTH response induced by IL-β, which is released during infectious and inflammatory conditions, is also attenuated after chronic exercise. Since both infection and exercise are stresses, it is interesting that, after chronic exercise, a cross-adaptation seems to occur in ACTH responses induced by infectious and physical stresses. Furthermore, since prostaglandins seem to be involved in the ACTH response induced by IL-1, this adaptation may be due to a reduction in prostaglandin synthesis and/or to an enhancement of the rate of prostaglandin degradation.

The contribution of prostaglandins in stress-induced responses has not been well established. However, Kluger, O'Reilly, Shope & Vander (1987) have recently shown that stress-induced hyperthermia is attenuated by pre-treatment with an inhibitor of prostaglandin synthesis, such as salicylate or indomethacin. Moreover, as is well known, fever is also suppressed by these inhibitors. These data support the hypothesis that prostaglandins play an important role in stress-induced responses. Furthermore, Cannon & Kluger (1983) suggested that endogenous pyrogen-like activity increases during exercise. Therefore, we must consider the possible involvement of endogenous pyrogen in the induction of prostaglandins during stressful conditions.

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